

Please cancel claims 8-30.

Please add the following claims:

31. (Amended) A method for directing the biosynthesis of specific macrolide polyketide analogs by genetic manipulation of a polyketide-producing microorganism, said method comprising the steps of:

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- (1) isolating a macrolide polyketide biosynthetic gene-containing DNA sequence;
  - (2) identifying enzymatic activities associated within said gene-containing DNA sequence;
  - (3) introducing one or more specified changes into said gene-containing DNA sequence which codes for one of said enzymatic activities resulting in an altered DNA sequence;
  - (4) introducing said altered DNA sequence into a polyketide-producing microorganism to replace the original sequence;
  - (5) growing a culture of the altered microorganism under conditions suitable for the formation of the specific macrolide polyketide analog; and
  - (6) isolating said specific macrolide polyketide analog from the culture.

32. The method of claim 31 wherein said macrolide polyketide biosynthetic gene-containing DNA sequence is derived from the polyketide synthase for the production of 6-deoxyerythronolide B (6 deB).

33. The method of claim 31 wherein the altered nucleotide sequence is derived from the rapamycin PKS.

34. A method for directing the biosynthesis of a specific macrolide polyketide analog which method comprises the steps:

- A3  
cm<sup>it</sup>
- (1) providing a nucleotide sequence encoding a macrolide polyketide synthase (PKS);
  - (2) identifying at least one region of said nucleotide sequence that encodes an enzymatic activity;
  - (3) introducing one or more specified changes into said region resulting in an altered nucleotide sequence;
  - (4) introducing said altered nucleotide sequence into a microorganism;
  - (5) growing a culture of said microorganism under conditions suitable for the formation of the specific macrolide polyketide analog; and
  - (6) optionally isolating the specific macrolide polyketide analog from culture.

35. The method of claim 34 wherein said nucleotide sequence of step 1 encodes at least two modules of the erythromycin PKS.

36. The method of claim 34 wherein said nucleotide sequence of step 1 encodes a complete macrolide PKS.

37. The method of claim 36 wherein said nucleotide sequence of step 1 encodes a complete erythromycin PKS.

38. The method of claim 34 wherein said introducing of step 3 comprises deleting said at least one region.

39. The method of claim 34 wherein said introducing of step 3 comprises replacing said at least one region with a corresponding region of a nucleotide sequence encoding an enzymatic activity of a different macrolide PKS.

40. The method of claim 39 wherein said region encodes an enzymatic activity selected from the group consisting of ketosynthase (KS) activity; acyl transferase (AT) activity; ketoreductase (KR) activity; dehydratase (DH) activity; and enoyl reductase (ER) activity.

41. The method of claim 34 wherein said introducing of step 3 comprises mutating said at least one region.

42. The method of claim 34 wherein the macrolide PKS of step 1 is selected from the group consisting of rapamycin, avermectin, FK-506, FR-008, monensin, rifamycin, soraphen-A, spinocyn, squalestatin, and tylosin.

43. The method of claim 39 wherein the corresponding region is derived from a PKS selected from the group consisting of rapamycin, avermectin, FK-506, FR-008, monensin, rifamycin, soraphen-A, spinocyn, squalestatin, and tylosin.

#### REMARKS

Claims 1-7 were subject to a restriction requirement in the parent application herein and represent a non-elected invention. New claims 31-43 represent more detailed descriptions of the invention of claims 1-7. Support for these claims is found, for example, on page 6 of the specification line 16 - page 7, line 18; support for claims 42 and 43 is found on page 5, line 28 - page 6, line 2; support for specific embodiments of the invention claimed is found in example 1-5. No new matter has been added and entry of the amendment is respectfully requested.

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